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THE EFFECT OF IMMOBILIZATION ON CATECHOLAMINE STORES IN SOME CENTRAL AND PERIPHERAL TISSUES OF THE RAT

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Catecholamines in some central (hypothalamus, Corpus striatum) and peripheral tissues (suprarenal glands, heart auricles) were determined after periods of immobilization of rats lasting from 30 to 240 min. It was found that sufficiently long immobilization produced significant depletion of both central and peripheral catecholamine stores. The strongest and the quickest depletion occurred in the suprarenal gland (adrenaline). Highly significant depletion of noradrenaline and dopamine in the hypothalamus was obtainde. (The stores of dopamine in the Corpus striatum and of noradrenaline in the heart auricles were the most resistant to immobilization stress. The degree of catecholamine depletion in all investigated structures depended on the duration of immobilization, i. e. on the severity of the stress. The longer the immobilization, the stronger the depletion of catecholamines. The experiments indicated that after immobilization lasting for more than 30 min the release of catecholamines both from central and peripheral tissues was stronger than the processes of synthesis and reuptake, thus leading to progressive depletion of catecholamine stores, at least when immobilization lasted for up to 240 min.

Key wards: catecholomine, tissues, dopamine, noradrenaline

INTRODUCTION

It has been already found that immobilization produces a significant increase in the plasma catecholamine concentration in rats (Kvetnansky et al., 1978; 1984). Changes in the brainstem catecholamine store occur even after five min restraint stress (Lachuer et al., 1991). Changes in plasma catecholamine levels in rats also occur after long-term exposure to immobilization stress, 40 x for 150 min (Kvetnansky et al., 1984), or only 60 min (Zukowska-Grojec et al., 1988).

Immobilization stress, in six 15 min intermittent periods or single 90 and 180 min periods continuously, produced significant increases in hypothalamic

MHPG (3-methoxy-4-hydroxy-phenylethyleneglycol) (Shimizu et al. 1990) and also noradrenaline in several other brain regions (thalamus, anterior cerebral cortex, *Locus coeruleus,* basal ganglia) (Ida et al., 1990).

It was therefore of interest to compare the effects of long lasting immobilization on catecholamine stores in some brain regions, the suprarenal glands and heart auricles.

MATERIALS AND METHODS

The experiments were carried out on male normotensive Wistar rats (300 - 320 g), bred and kept under ordinary laboratory conditions with water and food ad libitum.

The rats were immobilised by fixing them to a board. All four limbs of the animal were fixed to the board by sticking tape. The head was also held by a metal loop over the neck area of the animal, thus limiting the motion of the head (Kvetnansky and Mikulaj, 1970; Kventansky et al., 1978). Immobilization lasted 30, 60, 90, 120 and 240 min, respectively. After the period of immobilization the animals were decapitated. *Corpus striatum,* hypothalamus, heart auricles and adrenals were dissected out immediately after decapitation and immersed in cold (4^0C) 0.1N $HClO_4$ (0.3 μg of tissue per 30 ul of 0.1N $HC10_4$). The tissue was then homogenized (10.000 rotations per min for 15 min). The supernatant (30 μl) was used for analysis.

Catecholamines in tissues were determined using a modified method of Da Prada and Zürcher (1976), Weise and Kopin (1976) and Peuler and Johnson (1977). The principle of the method was to convert the catecholamines present into the corresponding O-methyl derivatives by means of purified catechol-O-methyl-transferase (COMT) in the presence of S-adenosyl-l-(^{3}H-methyl)-methionine. The O-methyl derivatives are extracted and oxidized into ^{3}H-vanilline. The activity of this substance was measured using a liquid scintillation counter (Packard).

The statistical significance of the results was tested by Student's t-test.

The following substances were used: S-adenosyl-1-(^{3}H-methyl) - methionine (Amersham) and EGTA (Sigma). COMT was prepared according to the method of Axelrod and Tomchik (1958).

RESULTS

Catecholamine levels in the hypothalamus and Corpus striatum after immobilization from 30 to 240 min. - Two typical structures in the central nervous system containing catecholamines are the hypothalamus and *Corpus striatum.* Both noradrenaline and dopamine are present in the hypothalamus, but only dopamine in the *Corpus striatum.* A short lasting immobilization of 30 min did not produce any significant change in catecholamine levels in these central structures. Even immobilization for 60 min did not produce a significant change in the level of dopamine in the *Corpus striatum.*

Meanwhile, immobilization of 60 min and more, up to 240 min, produced a highly significant depletion of noradrenaline and dopamine in the hypothalamus. Dopamine stores in the *Corpus striatum* were more resistant to immobilization. Only periods of immobilization from 90 to 240 min produced significant and highly significant decreases in the catecholamine stores in the *Corpus striatum.*

The results are presented in Table 1.

Table 1. Concentrations of noradrenaline and dopamine in the *hypothalamus* and of dopamine in the *Corpus striatum* (in μg/g of fresh tissue) after immobilization of rats for periods of time ranging from 30 to 240 min. The number of experiments in indicated in parentheses. The values represent means ± s.e.m.

Duration of immobilization (in min)	Catecholamine level (μg/g)		
	Hypothalamus		Corpus striatum
	Noradrenaline	Dopamine	Dopamine
0 (Controls)	2.09±0.09 (12)	0.79±0.03 (12)	8.66±0.60 (14)
30	1.79±0.20 (3)	0.73±0.09 (3)	7.96±0.67 (3)
60	1.72±0.08** (10)	0.62±0.03*** (10)	7.46±0.30 (10)
90	1.39±0.08*** (10)	0.48±0.04*** (10)	7.37±0.32* (11)
120	1.21±0.09*** (11)	0.32±0.02*** (11)	6.47±0.39** (10)
240	0.47±0.05*** (12)	0.20±0.01*** (13)	4.83±0.34*** (11)

In comparison to the control values: * $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Catecholamine levels in the suprarenal glands after immobilization from 30 to 240 min. - Even a relatively short period of immobilization (30 min) produced a highly significant decrease in the amount of adrenaline in the suprarenal gland of the rat. Meanwhile, the same period of immobilization did not affect significantly the amount of noradrenaline in the suprarenals.

Contrary to this, all periods of immobilization from 60 min to 240 min produced a drastic depletion of both adrenaline and noradrenaline in the suprarenal glands. After immobilization of 240 min only 3.36% from the control value of adrenaline remained in the gland. The same period of immobilization depleted noradrenaline level to 31.2% in comparison to the control value.

The results are presented in Table 2.

The amount of noradrenaline in the heart auricles after immobilization from 30 to 240 min. - Even 60 min of immobilization did not affect noradrenaline stores in the heart auricles. Periods of immobilization lasting from 90 to 240 min produced a significant depletion of noradrenaline stores in the auricles. Never the less, the degree of depletion reached only 56% in comparison to the control value, as found after 240 min of immobilization.

The results are presented in Figure 1.

Table 2. — Concentrations of adrenaline and noradrenaline in the *suprarenal glands* of rats (in μg/suprarenal gland) after immobilization for periods of time ranging from 30 to 240 min. The number of experiments is indicated in parentheses. The values represent means ± s.e.m.

Duration of immobilization (in min)	Adrenaline	Noradrenaline
0 (Controls)	20.21±1.21 (11)	2.50±0,15 (11)
30	12.19±1.30*** (3)	1.95±0.24* (3)
60	5.64±0.32*** (11)	1.26±0.08*** (12)
90	2.98±0.34*** (10)	1.89±0.04*** (10)
120	2.29±0.16*** (10)	0.79±0.04*** (10)
240	0.68±0.06*** (9)	0.78±0.06*** (9)

In comparison to the control values: * $P < 0.05$
*** $P < 0.001$

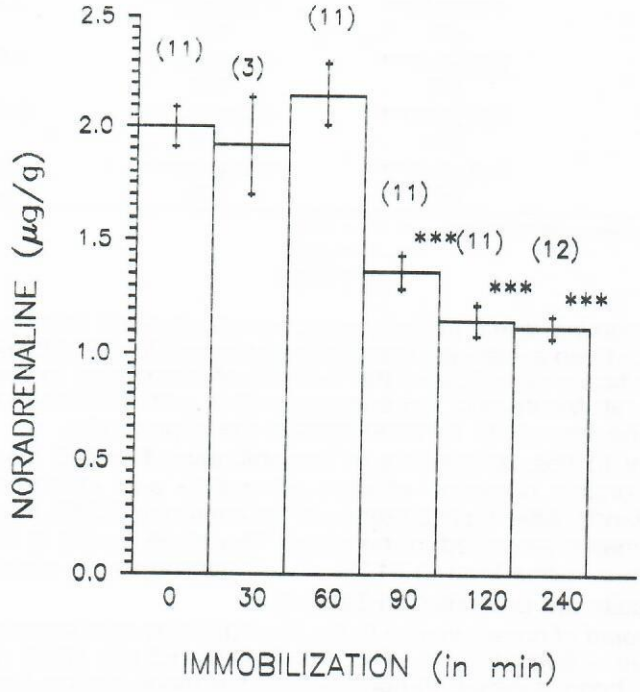

Figure 1. Concentration of noradrenaline (in μg/g of fresh tissue) in the auricles of the rat after periods of immobilization ranging from 30 to 240 min. The columns represent the means ± s.e.m. The number ef experiments is indicated in parentheses above the columns. *** $P < 0.001$.

DISCUSSION

It was found in the present experiments that sufficiently long immobilization produced a significant depletion of both central and peripheral catecholamine stores. The strongest and the quickest depletion occurred in adrenaline stores in the suprarenal gland. The longest period of immobilization of the rat used in these experiments (240 min) decreased the level of adrenaline in the suprarenal gland to only 3.36% of the control value. Depletion of noradrenaline stores in the adrenals was less pronounced. Thus, the longest period of immobilization depleted noradrenaline stores down to 31.2% of controls.

Noradrenaline stores in the hypothalamus were depleted down to 22.4% after a period of immobilization lasting 240 min. Similarly, dopamine stores in the hypothalamus decreased to 25.3% in comparison to the control value, when immobilization lasted for 240 min.

The most resistant to immobilization stress were the stores of dopamine in the *Corpus striatum* and noradrenaline in the heart auricles. Thus, the longest immobilization used in these experiments depleted noradrenaline in the heart auricles only to 56% in comparison to the control value. Dopamine in the *Corpus striatum* was decreased to almost the same relative level (55.7%) after immobilization for 240 min.

The data obtained in the present experiments indicate that the degree of catecholamine depletion in central and peripheral tissues depends on the duration of immobilization, i. e. on the severity of the stress. However, the same stressor can produce changes in the catecholamine levels to various degrees in different tissues.

Acute immobilization stress has been known to produce a significant increase in the plasma catecholamine levels (Kvetnansky et al., 1978; Stepanović et al., 1988, 1989). Restraint stress of very short duration (5 min) has been found to increase the activity of the central noradrenergic cells, as measured by an increase of DOPAC, a metabolite of adrenaline and noradrenaline (Lachuer et al., 1991). Similarly, immobilization for 90 or 180 min continuously, produced significant increases in hypothalamic MHPG, also a metabolite of catecholamines (Shimizu et al., 1990). Noradrenaline synthesis was also stimulated by immobilization stress after 30 min, as measured by accumulation of DOPA in the hippocampus, a prevalently noradrenergic area in the rat, after which period accumulation of DOPA declined. Similarly, the same type and duration of immobilization produced an accumulation of DOPA in the striatum, a prevalently dopaminergic area (Algeri et al., 1988). If immobilization lasts longer, as in the present experiments, then the release of catecholamines both from central and peripheral stores produces a progressive depletion. The longer the immobilization, the stronger the depletion of catecholamines. Contrary to this, a long-term immobilization stress (40 times 150 min every day) increased the catecholamine stores in tissues, evidently by enhanced biosynthesis rendered possible by increased the availability of catecholamine synthesizing enzymes (Kvetansky et al., 1984).

Immobilization of rats for just 1 and 3 min, or only gentle handling of the animals, rapidly increased the plasma levels of catecholamines, and DOPA, as well as of metabolites of noradrenaline and dopamine, indicating very rapid increases in the synthesis, release, reuptake and metabolism of catecholamines (Kvetnansky et al., 1992). Evidently, during these forms of stress increases in the turnover of endogenous catecholamines are matched by enhanced catecholamine synthesis. Our experiments indicate that after immobilization lasting for more than 30 min the release of catecholamines from both central and peripheral tissues is stronger than the processes of synthesis and reuptake, thus producing a progressive depletion of catecholamine stores. Never the less, the processes of turnover may be sufficiently high to keep the plasma levels of catecholamines increased as a response to immobilization stress.

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EFEKT IMOBILIZACIJE NA DEPOE KATEHOLAMINA U NEKIM CENTRALNIM I PERIFERNIM STRUKTURAMA PACOVA

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SADRŽAJ

U ovom radu su određivani kateholamini u nekim centralnim (hipotalamus, *Corpus striatum)* i perifernim tkivima (nadbubrežna žlezda, srčane pretkomore) posle perioda imobilizavcije pacova u trajanju od 30 do 240 minuta. Dovoljno duga imobilizacija prouzrokuje značajno smanjenje kako centralnih tako i perifernih depoa kateholamina. Najjače i najbrže smanjenje nastaje u nadbubrežnoj žlezdi (adrenalin).

Vrlo značajno osiromašenje u noradrenalinu i dopaminu nađeno je i u hipotalamusu. Najotporniji prema imobilizaciji su depoi kateholamina u *Corpus striatum* (dopamin) i srčanim pretkomorama (noradrenalin). Stepen osiromašenja kateholaminskih depoa u svim istraživanim strukturama zavisio je od trajanja imobilizacije, tj. od jačine stresa. Što je duža imobilizacija, jače je i osiromašenje kateholaminskih depoa. Izvedeni eksperimenti ukazuju da posle imobilizacije koja traje duže od 30 minuta oslobađanje kateholamina iz centralnih i perifernih depoa je jače nego što su aktivni procesi sinteze i preuzimanja, što vodi do progresivnog osiromašenja kateholaminskih depoa.